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Anemia Associated with Impaired Erythropoietin Secretion after Allogeneic Stem Cell Transplantation: Incidence, Risk Factors, and Response to Treatment

Anna Gaya, MD, Alvaro Urbano-Ispizua, MD, PhD, Francesc Fernández-Avilés, MD, PhD, Olga Salamero, MD, Josep María Roncero, MD, Montserrat Rovira, MD, PhD, Carmen Martínez, MD, PhD, Carme Talarn, MD, Miquel Granell, MD, Enric Carreras, MD, PhD, Emili Montserrat, MD, PhD

Department of Hematology, Institute of Hematoncology, Hospital Clínic of Barcelona; IDIBAPS, University of Barcelona, Barcelona, Spain

Correspondence and reprint requests: Francesc Fernández-Avilés, MD, PhD, Department of Hematology, Hospital Clínic of Barcelona, Villarroel 170, 08036 Barcelona, Spain (e-mail: ffernand@clinic.ub.es).

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ABSTRACT

After allogeneic stem cell transplantation (allo-SCT) some patients develop persistent anemia in association with an inadequate erythropoietin (Epo) secretion. We determined the frequency and risk factors for this complication and the response to treatment with erythropoiesis stimulating proteins (ESP). Of 83 evaluable allo-SCT patients, 63 (76%) developed persistent anemia at a median of 34 (range: 30-244) days after allo-SCT. Forty-one (49%) patients had anemia considered as primary, and in all of them inadequate serum Epo levels (median 43.3, range: 2.5-134, mU/mL) were found. A high creatinine level during the first month after allo-SCT was associated with primary anemia (relative risk [RR] 2.5, $P = .01$). Of the 41 patients, 35 received ESP. Transfusion independence and an Hb level higher than 10 g/dL was achieved in 29 of 30 (97%) evaluable patients. Median ferritin levels at the beginning and at the end of the ESP treatment was 1628 (range: 168-5208) and 805 (range: 14-7443) ng/mL, respectively ($P = .04$). In conclusion, anemia associated with impaired Epo secretion after allo-SCT is more frequent than usually recognized and it is associated to early postransplantation renal damage. This complication easily reverts with ESP, which seems to contribute to reduce iron overload.

KEYWORDS

Allogeneic stem cell transplantation • Anemia • Erythropoietin • Renal dysfunction

INTRODUCTION

Persistent anemia is common after allogeneic stem cell transplantation (allo-SCT), with some patients requiring red blood cell transfusions for as long as 1 year [1]. This is of medical concern because repeated transfusions are associated with iron overload and an increased risk of infections [2-5]. The etiology of the anemia after allo-SCT is multifactorial, but inadequate stimulus of erythroid progenitors by erythropoietin (Epo) seems to play an important role [1]. Thus, although serum Epo levels are increased within the first month after transplantation, Epo secretion is impaired afterward, resulting in inappropriately low levels for the degree of anemia for up to 6 months or 1 year [1,6-10]. Although impaired Epo secretion from allo-SCT was described 15 years ago, the incidence of anemia because of this cause has not been fully investigated.

Epo is synthesized by peritubular interstitial cells of the kidney in response to hypoxia [11-13]. The number of Epo-producing cells in the kidney has been shown to increase exponentially in response to anemia [14]. Cyclosporine A (CsA) is associated with tubular nephrotoxicity, and significantly impairs the ability of anemic patients to mount an adequate Epo response to anemia [1,15]. However, not all individuals receiving CsA develop anemia. Risk factors that contribute to anemia associated to an impaired Epo secretion after allo-SCT have not been identified.

Several groups have used erythropoiesis stimulating proteins (ESP) to accelerate erythropoietic

engraftment after allo-SCT [16-21]. However, its clinical usefulness is controversial, because in most clinical trials Hb levels were modestly improved by ESP and transfusion requirements were only marginally decreased [17,18]. These disappointing results might be explained by the fact that ESP was given from days 1 through 30 to day 55 or until erythroid engraftment, a period with a marked endogenous Epo release [1], and because patients received ESP regardless of whether they were anemic or not. A more rational use of ESP after allo-SCT has been only anecdotally reported [19].

Against this background, we investigated the effectiveness of ESP administration in patients having received an allotransplantation, with persistent primary anemia beyond day +30 and with demonstrated inadequate Epo secretion. We also looked for the incidence and risk factors associated with this complication and tried to quantify the benefits of the individualized utilization of ESP after allo-SCT.

PATIENTS AND METHODS

Patients

Between May 2002 and February 2005, 87 patients with hematologic diseases were submitted to allo-SCT in our institution. All of them received CsA as GVHD prophylaxis. Of them, 4 patients had a postransplantation survival shorter than 30 days and were excluded from this study. Detailed clinical characteristics of the 83 evaluable patients are listed in Table 1. The Hospital Clinic Ethical Committee approved the study and all patients signed a written informed consent.

Table 1. *Patient's Description*

Patient's Description	n = 83
Age (years), median (range)	41 (19-64)
Sex (M/F), n (%)	47/36 (56.6/43.4)
ABO compatibility, n (%)	
Compatible	42 (50.6)
Minor incompatibility	23 (27.7)
Major incompatibility	15 (18.1)
Combined major/minor incompatibility	3 (3.6)
Creatinine pre allo-SCT (mg/dL), median (range)	1.0 (1-2)
Stage of disease, n (%)	
Early	35 (42.2)
Advanced	48 (57.8)
Conditioning regimen, n (%)	
Myeloablative	56 (67.5)
TBI	49 (59)
No TBI	7 (8.5)
RIC	27 (32.5)
TBI	3 (3.6)
No TBI	24 (28.9)
Donor, n (%)	
HLA-identical sibling	57 (68.7)
Unrelated	26 (31.3)
Matched	19 (22.9)
Mismatched	7 (8.4)
Stem cells source, n (%)	
PB	69 (83.1)
BM	13 (15.7)
PB + BM	1 (1.2)
$CD34^+$ cell dose ($\times 10^6$/kg body weight), median (range)	4.2 (1-16)
AgCMV+ before day + 90, n (%)	34 (40.9)
Ganciclovir	14 (16.9)
Foscarnet	11 (13.2)
Valganciclovir	1 (1.2)
Ganciclovir + Foscarnet	7 (8.4)
No treatment	1 (1.2)

M indicates male; F, female; TBI, total body irradiation; RIC, reduced-intensity conditioning; PB, peripheral blood; BM, bone marrow.

Definitions

Patients with acute leukemia in first remission, chronic myelogenous leukemia (CML) in the chronic phase, and other hematologic malignancies in remission were classified as being in early stage. Patients with acute leukemia in the second or subsequent remission, multiple myeloma (MM), or other hematologic diseases in relapse were considered as in advanced stage [22]. Persistent anemia was considered when patients presented with hemoglobin concentration (Hb) lower than 10 g/dL beyond 30 days of transplantation, with or without RBC transfusion need. Persistent anemia was considered as secondary when an evident etiology (hemorrhage, graft failure, hemolysis, relapse of the hematologic disease, thrombotic microangiopathy, or severe infection) was present. Anemia was considered as primary when no evident cause for it was found. Inadequate Epo secretion was defined by either graphic or quantitative method (Epo <100 mU/mL in patients with Hb <10 g/dL) [23]. Renal function of each patient was evaluated by taking the maximum creatinine level during the first month of the transplantation and by the glomerular filtration calculated by Cockcroft-Gault formule (GF = [(140 − age) × kg body weight]/(72 × creatinine [mg/dL]) (×0.85 in females). Iron overload was defined as a ferritin level >1000 ng/mL [24,25].

Laboratory Analyses

Complete blood counts were determined in ADVIA 120 and 2120 autoanalyzers (Bayer Diagnostics, Barcelona, Spain). Serum Epo level was measured by commercially available ELISA (R&D Systems, Minneapolis, MN), with normal values ranging from 4 to 20 mU/mL. Ferritin level was measured by the immunoassay method in an ADVIA Centaur autoanalyzer (Bayer Diagnostics).

ESP Agents

The first 11 consecutive patients received epoietin α (Eprex®, Janssen-Cilag, or Epopen®, Pensa) at a standard dosage of 10,000 IU 3 times weekly, subcutaneously,

and the following 24 patients received darbepoietin α (Aranesp®, Amgen, Thousand Oaks, CA) at a standard dosage of 150 μg per week, subcutaneous. The only reason for changing epoietin to darbepoietin was the more convenient administration of darbepoietin α (once per week) compared to epoietin α (3 times per week). The objective of the treatment was to increase the Hb level over to 11 g/dL, and when raised to a level of ≥13 g/dL, the ESP treatment was interrupted.

Evaluation of Response to ESP

A major response was defined as an increment >2 g/dL in the Hb level, and by achievement of Hb level >10 g/dL without RBC transfusion needed, both within 4 weeks of starting the treatment. A minor response was considered when only 1 of these 2 criteria was fulfilled [19,26].

Statistical Method

The study was designed to identify the incidence and factors associated to primary anemia, to evaluate the degree of response to ESP, and to determine whether ESP treatment mobilizes iron overload. Characteristics considered to be associated with primary anemia were age, sex, ABO compatibility between patient and donor, maximum creatinine level between 1 and 30 days after allo-SCT, stage of disease (early versus advanced), preparative regimen (myeloablative versus reduced intensity (RIC), total body irradiation (TBI), number of $CD34^+$ cells infused, source of stem cells, type of donor (HLA-identical sibling or unrelated), presence of acute GVHD (aGVHD) (0-I versus II-IV), positive CMV antigenemia and its treatment (either ganciclovir or foscarnet were used depending on the presence renal disfunction or cytopenia, respectively), days to reach absolute neutrophil count (ANC) $>0.5 \times 10^9$/L, and days to achieve platelet count $>50 \times 10^9$/L. Descriptive analyses are reported as frequencies and percentages, median, and range. Pearson's chi-square (χ^2) test, Fisher's exact test, or Student's test were used to compare 2 groups. All reported P values are 2 sided, and a significance level of $\alpha = 0.05$ was used. All prognostic variables in the univariate analysis with a value of $P < .1$ were included for the multivariate analysis to ensure that each characteristic was individually significant. The multivariate analysis was performed using the logistic regression model. Statistical studies were performed by mean of SPSS 12.0 version statistical software.

RESULTS

Incidence of Primary Anemia and Impaired Epo Secretion

Sixty-three of the 83 (76%) patients presented with persistent anemia at a median of 34 (range: 30-244) days after allo-SCT. In 22 (27%) cases the anemia was secondary to transplantation-related complications: hemorrhage (n = 10, 15.8%), graft failure (n = 3, 4.8%), relapse (n = 3, 4.8%), thrombotic microangiopathy (n = 3, 4.8%), severe infection (n = 2, 3.2%), and hemolysis (n = 1, 1.6%). Secondary anemia was evident at a median of 38 (range: 30-158) days post-transplantation. In 41 (49%) patients anemia was considered to be primary, and it was apparent at a median of 33 (range: 30-244) days, and in all of them serum Epo level was inadequately low, with a median of 43.3 (range: 2.5-134) mU/mL. One patient, having a serum Epo level of 134 mU/mL with an Hb of 7.5 g/dL, was considered to have an inadequate Epo response by the graphical method [23].

Factors Associated with Primary Anemia

We analyzed the 41 patients that presented primary anemia, with (n = 27) or without (n = 14) RBC transfusion requirements. In the univariate analysis the variables associated with primary anemia were maximum creatinine level from 1 to 30 days after allo-SCT ($P = .001$), low glomerular filtrate from 1 to 30 days after allo-SCT ($P = .008$), positive cytomegalovirus (CMV) antigenemia ($P = .002$), independently of whether ganciclovir or foscarnet were used, and time to platelet recovery ($P = .006$) (Table 2). Twenty-eight of the 41 (68.3%) patients with primary anemia had a creatinine level during the first month after transplantation higher than the median (1.3 mg/dL) compared to only 4 of the 20 (20%) patients without anemia ($P = .0008$). ABO incompatibility, conditioning regimen, $CD34^+$ cell dose, and source of stem cells did not influence the incidence of primary anemia. In the multivariate analysis 3 independent factors predicted primary anemia: creatinine level after the first month of transplantation (relative risk [RR] 6.04, CI 1.4-25.9, $P = .02$), positive CMV antigenemia (RR 11.2, CI 2.1-60.3, $P = .005$), and delay in platelet engraftment (RR 6.4, CI 1.4-28.9, $P = .02$).

Response to ESP

Thirty-five of the 41 (85.3%) patients were treated with ESP. The remaining 6 patients with primary anemia did not receive ESP because of the appearance of severe comorbidities just before starting it. The first 11 patients received epoietin α (Eprex®, Janssen-Cilag, or Epopen®, Pensa) and the remaining 24 patients received darbepoietin alpha (Aranesp®, Amgen), because of its more convenient weekly administration. In 5 patients response was not evaluable because of early death (n = 2), discontinuation of follow-up because patients were referred to their original center (n = 2), and early relapse (n = 1). Out of the 30 remaining patients, 28 (93%) achieved a major and 1 (3.3%) a minor response, this last case attaining an Hb level superior to 10 g/dL without RBC transfusion. ESP treatment was associated with a rapid rise in Hb

Table 2. *Comparison of Characteristics of Patients without Anemia and Patients with Primary Anemia 30 Days after Allogeneic Transplantation*

Comparison of Characteristics of Patients without Anemia and Patients with Primary Anemia 30 Days after Allogeneic Transplantation	No Anemia (n = 20)	Primary Anemia (n = 41)	*P*
Age (years), median (range)	41.5 (20-61)	40 (19-61)	.3
Sex (M/F), n (%)	12/8 (60/40)	26/15 (63.4/36.6)	1.0
ABO compatibility, n (%)			
Compatible	11 (55)	18 (43.9)	.5*
Minor incompatibility	6 (30)	14 (34.1)	.7†
Major incompatibility	3 (15)	9 (22)	
Maximum creatinine 1-30 days post-allo-SCT (mg/dL), median (range)	1.1 (0.9-2.2)	1.6 (0.8-3.7)	.001
Glomerular filtrate 1-30 days post-allo-SCT (mL/min/)	80 (46-129)	60 (24-117)	.008
Stage of disease, n (%)			
Early	6 (30)	23 (56.1)	.06
Advanced	14 (70)	18 (43.9)	
Preparative regimen, n (%)			
Myeloablative	13 (65)	29 (70.7)	.7‡
TBI	11	24	.5§
No TBI	2	5	
RIC	7 (35)	12 (29.3)	
TBI	-	2	
No TBI	7	10	
$CD34^+$ cell dose ($\times 10^6$/kg body weight), median (range)	4.5 (1.1-10)	4.21 (0.9-16.45)	.8
Stem cells, n (%)			
PB	15 (75)	35 (85.4)	.4
BM	5 (25)	6 (14.6)	
Donor, n (%)			
HLA-identical sibling	15 (75)	26 (63.4)	.4¶
Unrelated	5 (25)	15 (36.6)	1.0⊥
Match	4 (20)	11 (26.8)	
Mismatch	1 (5)	4 (9.8)	
Acute II-IV GVHD, n (%)	2 (10)	13 (31.7)	.1
CMV+ Ag before day + 90, n (%)	3 (15)	24 (58.5)	.002
Median day (range) to reach ANC $>0.5 \times 10^9$/L	16 (11-24)	18 (0-33)	.2
Median day (range) to reach platelet count			
$> 20 \times 10^9$/L	12.5 (0-35)	18 (0-286)	.01
$> 50 \times 10^9$/L	17 (12-41)	25 (0-322)	.006

M indicates male; F, female; TBI, total body irradiation; RIC, reduced-intensity conditioning; PB, peripheral blood; BM, bone marrow; ANC, absolute neutrophil count; GVHD, graft-versus-host disease; CMV, cytomegalovirus.

*ABO compatibility versus ABO incompatibility.

†ABO compatibility + minor ABO incompatibility versus major ABO incompatibility.

‡Myeloablative regimen versus RIC.

§Conditioning with TBI versus conditioning without TBI.

¶HLA-identical sibling versus UD.

⊥HLA-identical sibling and match unrelated donor versus mismatch donor.

concentration, with Hb values being of 8.8 (range: 6.6-10.1) g/dL before ESP treatment and of 11.6 (range: 8.9-14.5) g/dL after 1 month of drug administration ($P = 6 \times 10^{-10}$) (Figure 1A), and with a rise in the reticulocyte count, from 85 (range: 19.5-209.4) $\times 10^9$/L to 138.4 (range: 34.1-494) $\times$ 10^9/L ($P = .008$). The proportion of patients requiring RBC transfusion before and after 1 month of treatment was 60% and 26.6%, respectively ($P = .02$) (Figure 1B). Full-dose treatment (epoietin α 10,000 IU 3 times per week; darbepoietin 150 μg per week) was discontinued after a median time of 42 (range: 18-230) days without further need of RBC transfusion. After this period ESP dosage was tapered to maintain an Hb level superior to 11 g/dL, so the median time duration of the complete treatment was 240 (26-981) days. A delayed response was observed in 2 patients; in both of them iron deficiency was detected and a rapid Hb response was observed after oral iron administration. Only these 2 patients received supplements of iron during ESP treatment. Of the whole group treated with ESP, only 1 (3.3%) case did not respond after 3 months of treatment. A possible explanation of the lack of response in this particular case is that a relapse of the hematologic disease was observed shortly thereafter (Table 3). One patient presented with a deep vein thrombosis in the left leg while receiving ESP treatment concurring with an Hb level of 9.5 g/dL. Anticoagulant treatment with low molecular weight heparin was started with total resolution of the thrombosis.

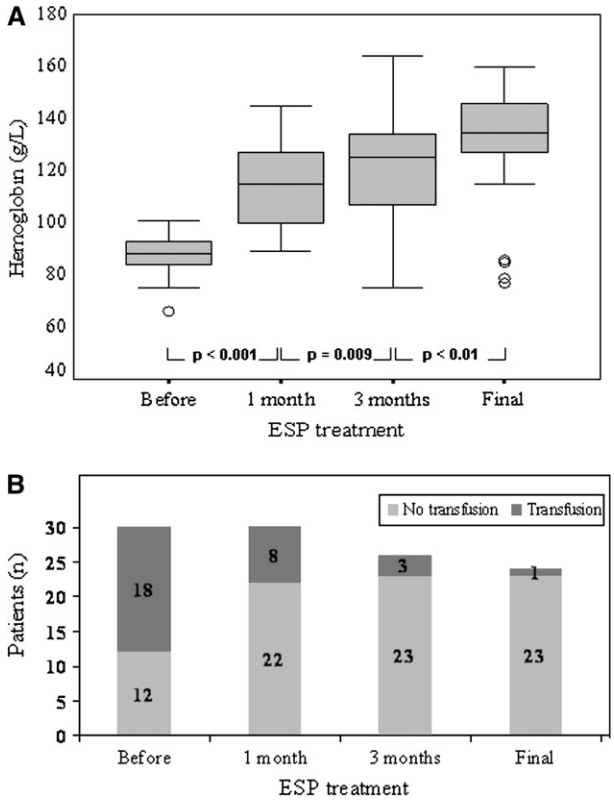

Figure 1. (A) Hb response to ESP treatment; (B) RBC transfusion requirements.

Ferritin Values before and after ESP Treatment

The median ferritin level before and after ESP administration was 1628 (range: 168-5208) ng/mL and 805 (range: 14-7443) ng/mL, respectively ($P < .04$) (Figure 2). The proportion of patients with a ferritin value superior to 1000 ng/mL before starting ESP was 78% compared to 50% at the end of the treatment ($P = .16$).

Table 3. *Characteristics and Response to ESP Treatment*

Time post allo-SCT to initiate ESP treatment, median (range) days	**83 (40-257)**
Pre ESP treatment RBC transfusion, n (%)	
No	**12 (40%)**
Yes	**18 (60%)**
Type of ESP, n (%)	
erythropoietin α	**11 (31%)**
darbepoietin α	**24 (69%)**
Response to ESP treatment, n (%)	**29/30 (97%)**
Major	**28**
Minor	**1**
No evaluable, n (%)	**5/35 (14.3%)**
ESP treatment duration, median (range) days	**240 (26-981)**
Full dose	**42 (18-230)**
Maintenance	**201 (23-952)**
Iron supplement, n (%)	**2/30 (6.7%)**

ESP indicates erythropoiesis stimulating proteins; RBC, red blood cells.

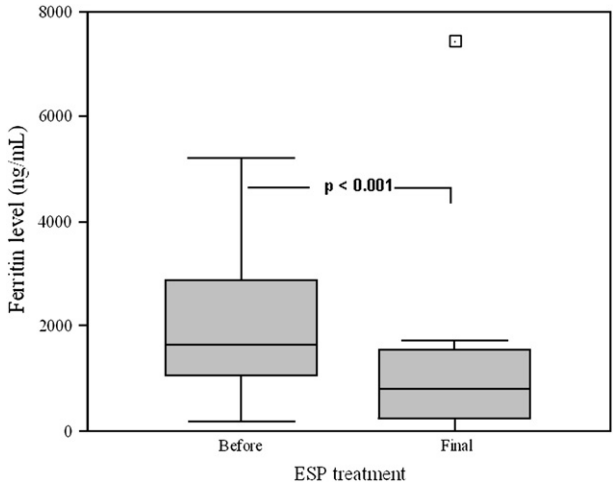

Figure 2. Decrease of ferritin level.

DISCUSSION

Anemia associated with impaired Epo secretion after allo-SCT is likely to be more frequent than usually recognized. In our study, half of the patients submitted to allo-SCT developed persistent anemia 1 month after transplantation, not associated to hemorrhage, graft failure, hemolysis, leukemic relapse, or severe infection. All these patients had inadequately low serum Epo levels, a well-known phenomenon after allo-SCT that has been linked to cytokines release and to the use of CsA [6,15,27-31].

IL-1 and TNF-α levels have been shown to be increased after allogeneic transplantation, as a result of acute or chronic inflammatory states, especially in relation to GVHD [27]. These cytokines suppress the marrow proliferative response by inhibiting Epo production and by blocking its action on erythroid precursor cells [28,29]. This mechanism would explain the association between CMV infection and primary anemia as found in this study. CMV infection induces the release of macrophage and T cell-derived cytokines that have been shown to inhibit the growth of erythroid progenitors in vitro [30,31]. Moreover, it has been also demonstrated that CMV infection causes defective Epo production [6].

Regarding the association of CsA and impaired Epo secretion, Vannucchi et al. [15] in 1991 found in a mouse model that serum Epo levels were constantly low in those CsA treated, a finding later corroborated in humans [6]. Although the inhibitory effect of CsA on Epo secretion was found to be independent from the drug dose and creatinine level [6,15], a subclinical kidney toxicity produced by CsA has been considered the cause of the impaired Epo production [15]. Allo-SCT may produce long-term renal dysfunction [32], renal damage occurring within the first 3 weeks after transplantation in most cases [32]. We selected this period of time for identifying renal dysfunction as

a potential causative factor of severe impaired Epo secretion and subsequent anemia. We found that renal damage during the first month after allo-SCT is an important factor for further developing persistent anemia, which is consistent with the notion that renal disease is almost always accompanied by a profound failure of the normal Epo response. Thus, 91.3% of patients with creatinine higher than the median (1.3 mg/dL) developed anemia compared with 56.8% of patients with normal creatinine level ($P = .0005$). It would appear that renal insufficiency worsens the already impaired Epo secretion because of CsA, impeding in these patients to mount an adequate erythropoietic response to anemia.

A number of different factors such as nephrotoxic antibiotics, anti-inflammatory and antihypertensive drugs, severe infections, and chemoradiotherapy may contribute to the occurrence of renal toxicity after allo-SCT, but CsA is a constant factor for this complication. In fact, the major limiting factors in the use of CsA are the acute and long-term toxic effects over the kidney [33,34]. In the short term, CsA induces a reversible reduction in renal blood flow and glomerular filtration rate [35]. In the long term, CsA treatment leads to irreversible renal failure characterized by extensive tubulointerstitial fibrosis [36]. The long-term effects of CsA toxicity can be attributed to sustained restriction of renal blood supply related to a decreased nitric oxide production in renal arterioles [37]. Notably, ESP reduces the consequences of oxidative stress and promotes proangiogenic actions. Recent reports, in which ESP were used to ameliorate renal failure, are encouraging [38-41]. Whether ESP administration for correcting anemia after allo-SCT will also be associated with improvement in renal function requires specific investigations.

Although the inappropriate Epo secretion in anemic patients after allo-SCT supports the use of ESP in these patients, controlled trials have shown little clinical benefit, including little reduction in transfusion requirements and poor cost-effective ratios [16-18,20]. Several reasons may explain these discouraging results. First, in most of these trials ESP was administered in the early postransplantation period, when in fact, there is high serum level of endogenous Epo [8]. Second, patients were treated independently of whether they were anemic or not, and regardless of whether the anemia was because of impaired Epo secretion or to other causes [21]. Third, the ESP dose administered was very high, making it difficult to justify their use on economic grounds. A more rational use of ESP after allo-SCT is mandatory, especially considering that they can be associated to serious unexpected events such as thromboembolic episodes. Not surprisingly, EORTC guidelines state that for allo-SCT patients ESP are only recommended on an individual basis [42,43]. For ESP treatment, we selected patients with persistent anemia beyond the first month of the transplantation, with inadequate serum Epo level, and without other etiology for the anemia. Only a small cohort of patients from the literature has received a very similar tailored ESP administration [19]. Results from this series and those from Baron et al. [19] show that primary anemia after allo-SCT is exquisitely sensitive to ESP, with transfusion independence and complete correction of Hb being achieved in more than 95% of the patients. Our study confirms that the majority of patients requires only a short course of treatment to respond and that ESP can be discontinued in the majority of responders without requiring retreatment with ESP for recurrent anemia [19,21]. Of note, response to treatment was observed in all patients regardless of whether they had received a myeloablative or RIC regimen. This is in contrast to the finding of Baron et al. [44] that suggested that RIC transplantations are not associated with impaired Epo production.

Iron overload after allo-SCT is frequently observed in heavily transfused patients [2,45-48], and is associated with a higher probability of transplantation-related mortality, mainly because of more frequent organ toxicity and severe infections [2,3,49-52]. A safe and simple measurement of ferritin level provides an approximation of the total body iron content [50,53]. Phlebotomy removes the excess of iron accumulated but it is associated with anemia [50]. In contrast, ESP administration might decrease iron overload by both avoiding repeated transfusions and by utilization of deposits of iron [19,53-55]. Indeed, we have observed that after ESP administration ferritin levels significantly decreased. A similar finding has been observed in healthy donors and in renal end-stage patients treated with ESP [54-56].

In conclusion, anemia associated with impaired Epo secretion after allo-SCT is more frequent than recognized and is mainly observed in patients with renal dysfunction during the first month after transplantation. Although the etiology of primary anemia after allo-SCT is multifactorial, and in addition to low Epo levels, poor graft function, GVHD, and viral infections may all contribute to reduce hemopoiesis, it is exquisitely sensitive to ESP, with transfusion independence and complete correction of Hb being achieved in more than 95% of the patients. An additional benefit of ESP administration in these patients is that it might contribute to reduce the incidence of iron overload by eliminating the need of transfusions and by mobilizing iron deposits. These results must be validated in larger numbers of patients.

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